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Role of the MexAB-OprM Efflux Pump of *Pseudomonas aeruginosa* in Tolerance to Tea Tree (*Melaleuca alternifolia*) Oil and Its Monoterpene Components Terpinen-4-ol, 1,8-Cineole, and α-Terpineol▽

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Using a series of efflux mutants of *Pseudomonas aeruginosa*, the MexAB-OprM pump was identified as contributing to this organism's tolerance to the antimicrobial agent tea tree (*Melaleuca alternifolia*) oil and its monoterpene components terpinen-4-ol, 1,8-cineole, and α-terpineol. These data show that a multidrug efflux system of *P. aeruginosa* can extrude monoterpenes and related alcohols.

Pseudomonas aeruginosa is an opportunistic pathogen notable for its high level of resistance to antimicrobial agents (11). The intrinsic multidrug resistance of *P. aeruginosa* is mediated by a combination of measures including an outer membrane with low permeability and the expression of tripartite multidrug efflux systems belonging to the resistance nodulation division family such as MexAB-OprM and MexXY-OprM (12, 29). Apart from antibiotics, the broad substrate range of the Mex efflux systems of *P. aeruginosa* also includes organic solvents, biocides, dyes, and cell signaling molecules (6, 19, 20, 28).

Derived from the Australian native plant *Melaleuca alternifolia* (Myrtaceae), tea tree oil (TTO) is an established broad-spectrum topical antimicrobial agent (2). Composed of terpene hydrocarbons, mainly monoterpenes, sesquiterpenes and their related alcohols, the composition of commercial TTO is regulated by an international standard (15). The oil has antibacterial, antiviral, and antifungal properties verified both in vitro and in a number of clinical trials (2). *P. aeruginosa* is less susceptible than most bacterial species to TTO, with MICs ranging from 1 to 8%, compared to <0.5% for other gram-negative bacteria (1, 2, 27). The activity of TTO and terpinen-4-ol, its main antimicrobial component, against *P. aeruginosa* is enhanced by the protonophore carbonyl cyanide *m*-chlorophenylhydrazone and the outer membrane chelator EDTA (9, 22). To elucidate the role of efflux pumps in tolerance to TTO, the susceptibility of a series of well-characterized efflux pump mutants of *P. aeruginosa* to TTO and five of its monoterpene components, terpinen-4-ol, 1,8-cineole, α-terpineol, γ-terpinene, and ρ-cymene, was examined.

The bacterial strains used in the present study are listed in Table 1. TTO (batch W/E504) was kindly provided by Australian Plantations Pty., Ltd., Wyrallah, New South Wales, Australia. The levels of the components determined by gas chromatography analysis and the range specified by the international standard (15) (shown in parentheses) were as follows: 40.3% (>30%) terpinen-4-ol, 19.7% (10 to 28%) γ-terpinene, 8.6% (5 to 13%) α-terpinene, 3.2% (0 to 15%) 1,8-cineole, 3.2% (1.5 to 5%) terpinolene, 3.1% (1.5 to 8%) α-terpineol, 2.4% (1 to 6%) α-pinene, 2.4% (0.2 to 12%) ρ-cymene, 1.6% (trace [tr] to 7%) aromadendrene, 1.2% (tr to 8%) δ-cadinene, 1.0% (0.5 to 4%) limonene, 0.5% (tr to 3%) globulol, 0.4% (tr to 1.5%) viridiflorol, and 0.1% (tr to 3.5%) sabinene. Terpinen-4-ol (100% pure) was provided by SNP Natural Products (Sydney, New South Wales, Australia). 1,8-Cineole (99% pure), α-terpineol (95% pure), and phe-arg-β-naphthylamide dihydrochloride (PAβND) were purchased from Sigma Chemical Company (St. Louis, MO). γ-Terpinene (97% pure) and ρ-cymene (99% pure) were purchased from Aldrich Chemical Company (Milwaukee, WI). Terpinen-4-ol and α-terpineol were selected on the basis of their marked antimicrobial activity (2). γ-Terpinene and ρ-cymene were selected since they are regarded as less antimicrobially active components, and 1,8-cineole was selected because it has modest activity and may be important for permeabilizing the outer membrane (3).

The MICs of TTO, terpinen-4-ol, 1,8-cineole, α-terpineol, γ-terpinene, and ρ-cymene were determined for the various efflux mutants and their wild-type strains by using the broth microdilution method described by the Clinical and Laboratory Standards Institute (7) with the following modification: all tests were performed in Mueller-Hinton broth supplemented with 0.002% Tween 80 (MHB-T). The compounds were tested over the range of 0.125 to 8% (vol/vol), and the MIC was the lowest concentration of TTO or component in which no growth was observed. Tests were performed at least three times, and a modal value was selected. In other MIC assays, PAβND, a known inhibitor of efflux in *P. aeruginosa* (8, 16),

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TABLE 1. Characteristics and TTO and component susceptibilities for efflux mutants and wild-type strains

Strain[a]	Relevant characteristics[b]	MIC (% [vol/vol])[c]				Reference(s)
		TTO	Terpinen-4-ol	1,8-Cineole	α-Terpineol	
PAO1	Prototroph, wild type	4	2–4	>8	>8	13, 21
PAO200	PAO1, Δ*mexAB-oprM*	0.5–1	0.5	2	1	30
PAO238	PAO1, Δ*mexAB-oprM* Δ*mexCD-oprJ*	0.5	0.25	0.5	<0.125	5
K1119	PAO1, Δ*mexAB-oprM*	1	0.25–0.5	2	0.5	21
K1521	PAO1, Δ*mexCD-oprJ*	2–4	2–4	>8	>8	10
K1523	PAO1, Δ*mexB*	1	0.25–0.5	4	1	14
K1525	PAO1, Δ*mexXY*	2–4	2	>8	>8	10
K1536	PAO1, *nfxB* (↑ *mexCD-oprJ*)	4	4	>8	>8	14
ML5087	Wild type	4	>8	>8	>8	26
K1110	ML5087, Δ*oprM*	1	0.5	2	0.5	21
K1112	ML5087, *nalB* (↑ *mexAB-oprM*)	2	>8	>8	>8	31
K1115	ML5087, Δ*mexAB-oprM* Δ*mexCD-oprJ*	1	0.25	2	0.25	21
K1121	ML5087, Δ*mexAB-oprM*	1	0.5	4	1	32
K1131	K1121, *nfxB* (↑ *mexCD-oprJ*)	1	0.25	2	0.25	32

[a] All strains were provided by Keith Poole, except PAO200 and PAO238, which were provided by Herbert Schweizer.
[b] Wild type, wild type with respect to status of multidrug efflux systems; ↑, overproduction.
[c] The MICs for γ-terpinene and ρ-cymene were >8% for all strains tested.

was added at 20 μg/ml. Differences in MICs determined for the various efflux mutants and their wild-type strains of ≥4-fold were considered significant.

The susceptibility of efflux pump mutants to TTO and terpinen-4-ol was also examined by using time-kill assays. A total of 10 ml of MHB was inoculated with one colony from a 24-h blood agar culture of *P. aeruginosa*, followed by incubation for 16 to 20 h at 35°C with shaking. This culture was diluted 1:100 with MHB-T to yield a 50-ml volume of suspension with approximately 10^7 CFU/ml. A 9.1-ml volume of cell suspension was dispensed into a 50-ml flask for each treatment or control. The treatments were 0.25 or 4% TTO or 0.125 or 1% terpinen-4-ol. At 5 min prior to time zero, 100 μl was removed, serially diluted in phosphate-buffered saline (PBS), and spread plated in duplicate onto predried nutrient agar plates. At time zero, a 1-ml aliquot of TTO, terpinen-4-ol, or PBS was added to the flask; the contents were then mixed for 20 s, and a 100-μl aliquot was removed for serial dilution at 0.5 min. The first 1:10 dilution of the aliquot was made in neutralizer (25) to arrest the effect of the oil or terpinen-4-ol. This first dilution was vortex mixed and left at room temperature for a minimum of 5 min to allow neutralization to occur. After this, the remaining dilutions were made in PBS. Flasks were incubated at 35°C with shaking for the 120-min duration of the assay. Samples were taken at 5, 10, 15, 20, 30, 60, and/or 120 min and were neutralized, diluted, and plated as at 0.5 min. Nutrient agar plates were incubated at 37°C overnight, and the viable count was determined.

Complementation of mutants deficient in MexAB-OprM was achieved by transforming with pRSP17 (pRK415::*mexAB-oprM*). The plasmids pRK415 and pRSP17 were extracted from *E. coli* strains K340 (17) and K1154 (31) (provided by Keith Poole), respectively. Cell suspensions of recipient strains of *P. aeruginosa* were prepared using a method based on that of Choi et al. (4). Cells were electroporated with plasmid in a Bio-Rad Gene Pulser II at 25 μF, 200 Ω, and 2.5 kV/cm for approximately 5 ms. Luria broth was added, and the cells were incubated in a sterile Bijou bottle at 37°C for 2 h with shaking. The mixture was plated onto a Luria agar plate containing 10 μg of tetracycline/ml, followed by incubation overnight at 37°C. A plasmid miniprep confirmed the presence of the respective plasmids in several transformants, and the MICs of TTO and terpinen-4-ol for the transformants and wild-type strains were determined by broth microdilution as described above, with 10 μg of tetracycline/ml added for transformants.

PAO200, K1119, and K1121, all MexAB-OprM$^-$, and the MexB$^-$ and OprM$^-$ strains K1523 and K1110, respectively, showed an increase in susceptibility to TTO, terpinen-4-ol, 1,8-cineole, and α-terpineol compared to wild-type strains (Table 1). This increase ranged from 4 to 8 times for TTO, 4 to >16 times for terpinen-4-ol, >2 to >4 times for 1,8-cineole, and 8 to >16 times for α-terpineol. Hyperexpression of MexAB-OprM in K1112 made no significant change in susceptibility to TTO, and any changes in component susceptibility were beyond the detection limits of the assay due to component immiscibility at >8% (vol/vol). In time-kill assays with MexAB-OprM$^-$ strain K1121, one-quarter of the TTO MIC for that isolate reduced viability by 90% compared to slight growth in the ML5087 wild type (Fig. 1A). Treatment with 4% TTO (the MIC for the ML5087 wild type) caused a drop in viability below the limit of detection of the assay (3×10^3 CFU/ml) within 20 min in K1121, while only a 90% decrease in viability was seen in ML5087 after 120 min (Fig. 1A). Treatment with sub-MIC concentrations of terpinen-4-ol killed >90% of the MexAB-OprM$^-$ strain K1121 after 120 min but allowed growth in ML5087 (Fig. 1B).

Complementation of PAO200 (MexAB-OprM$^-$) with pRSP17 restored the TTO and terpinen-4-ol susceptibility of the strain from 0.5% (vol/vol) to that of the PAO1 wild type (2% [vol/vol]). The addition of pRK415 alone did not change the susceptibility.

The addition of the efflux inhibitor PAβND to broth microdilution assays decreased the TTO and terpinen-4-ol MICs for PAO1 to levels similar to those in MexAB-OprM$^-$ (PAO200 and K1119) and MexB$^-$ (K1523) strains (Table 2). Interestingly, there were further increases in the susceptibility of these three strains to terpinen-4-ol when PAβND was

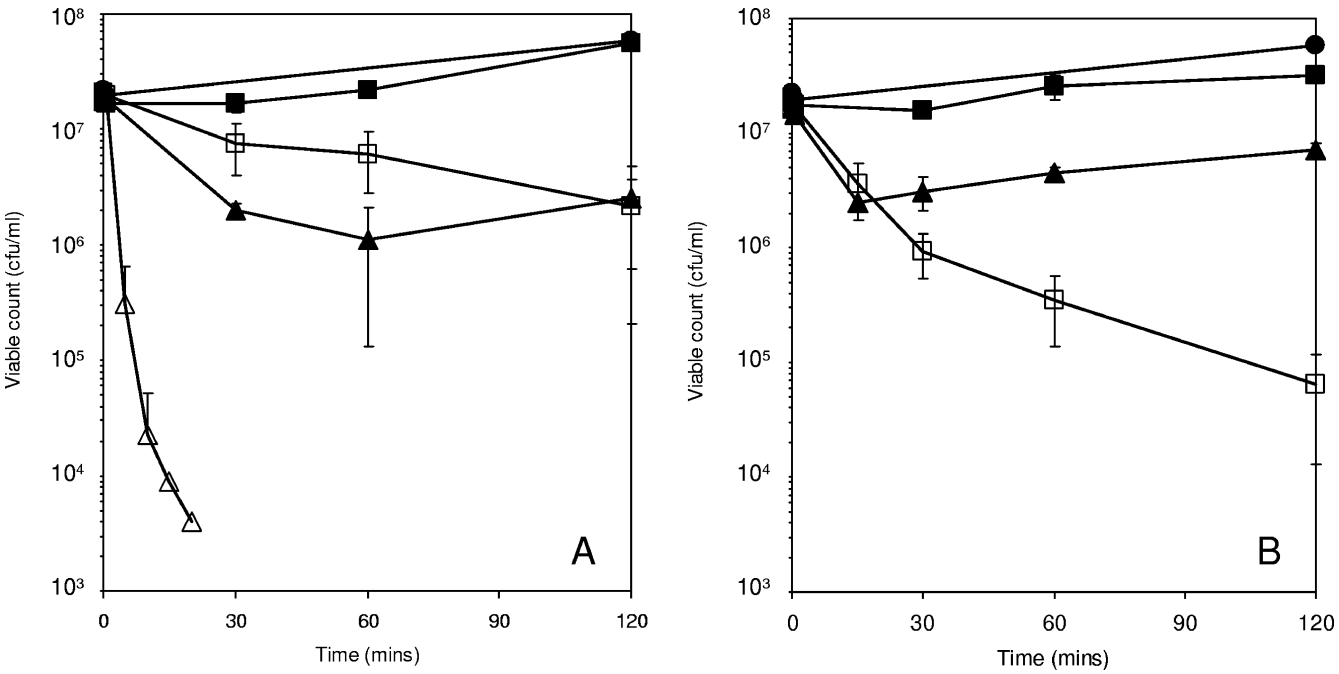

FIG. 1. Influence of the MexAB-OprM efflux system on the susceptibility of *P. aeruginosa* to TTO and terpinen-4-ol. (A) *P. aeruginosa* ML5087 treated with 0.25% (■) or 4% (▲) TTO. K1121 (MexAB-OprM$^-$) treated with 0.25% (□) or 4% (Δ) TTO. (B) *P. aeruginosa* ML5087 treated with 0.125% (■) or 1% (▲) terpinen-4-ol. K1121 treated with 0.125% (□) terpinen-4-ol. The MICs of TTO and terpinen-4-ol were 4 and >8%, respectively, for ML5087 and 1 and 0.5%, respectively, for K1121. A broth-only growth control for each isolate was included. ML5087 (●) is shown as representative of both strains. The error bars represent the standard deviations of at least three duplicate assays.

present, indicating that the MexAB-OprM pump may not be the only efflux system involved in tolerance to this component.

The concurrent loss of the MexAB-OprM and MexCD-OprJ pumps provided some evidence for a role for the MexCD-OprJ pump in terpene efflux. In PAO238 (MexAB-OprM$^-$ and MexCD-OprJ$^-$) the 1,8-cineole MIC was one-quarter that of the PAO200 (MexAB-OprM$^-$) strain, while the α-terpineol MIC dropped from 1 to <0.125%. The TTO and terpinen-4-ol MICs were not altered. Similarly, two- to fourfold reductions in MICs of 1,8-cineole and α-terpineol were seen in K1115 (MexAB-OprM$^-$ and MexCD-OprJ$^-$) compared to K1121 (MexAB-OprM$^-$). Again, the TTO and terpinen-4-ol MICs remained the same. It may be that the MexCD-OprJ pump effluxes 1,8-cineole and α-terpineol more efficiently than TTO or terpinen-4-ol since tolerance to the latter agents did not change. However, this is difficult to explain on the basis of physicochemical properties since α-terpineol and terpinen-4-ol have very similar aqueous solubilities and octanol-water partition coefficients (2). That aside, previous studies have shown an additive effect on drug resistance from the simultaneous expression of resistance nodulation division efflux pumps (18). It seems likely that interplay between the MexAB-OprM and MexCD-OprJ pumps may contribute to tolerance to some components of TTO, including 1,8-cineole and α-terpineol.

The MexXY pump usually requires the OprM component to export substrates, and so the loss of the MexAB-OprM pump may also cause a loss of function for MexXY (24). There was no change in the susceptibility of strain K1525 (lacking the MexXY components) compared to the wild-type strain (Table 1). K1523, which is deficient in MexB, and the two MexAB-

TABLE 2. Effect of PAβND on susceptibility to TTO and terpinen-4-ol in *P. aeruginosa* PAO1 and several efflux mutants

Strain	Relevant characteristics	MIC (%, vol/vol)			
		TTO	TTO plus PAβND	Terpinen-4-ol	Terpinen-4-ol plus PAβND
PAO1	Prototroph[a]	4	0.5–1	2–4	0.25
PAO200	PAO1, Δ*mexAB-oprM*	0.5–1	0.25–0.5	0.5	<0.125
K1119	PAO1, Δ*mexAB-oprM*	1	0.25–0.5	0.25–0.5	<0.125
K1521	PAO1, Δ*mexCD-oprJ*	2–4	1	2–4	0.25
K1523	PAO1, Δ*mexB*	1	0.5	0.25–0.5	<0.125
K1525	PAO1, Δ*mexXY*	2–4	1	2	0.25
K1536	PAO1, *nfxB*	4	0.5–1	1–≥8[b]	0.25

[a] Wild type with respect to the status of multidrug efflux systems.
[b] This was tested nine times, and the results were inconsistent. Therefore, a range is given. It is possible that terpinen-4-ol is a weak inducer, and a substrate, of the MexCD-OprJ pump.

OprM$^-$ strains PAO200 and K1119 had almost identical susceptibilities, indicating that this change in susceptibility was probably related solely to the loss of the MexAB-OprM pump and not to a loss of function of MexXY due to loss of the OprM component.

γ-Terpinene and ρ-cymene did not appear to be substrates of any of the pumps examined, although their low antimicrobial activities may have meant that changes to their MICs went undetected above the upper limit of the susceptibility test. Alternatively, we may not have examined the pumps for which they are substrates. Given that γ-terpinene has demonstrated activity against *P. aeruginosa* under certain circumstances (22, 23), the latter seems more likely.

Susceptibility to TTO and the components terpinen-4-ol, 1,8-cineole and α-terpineol was increased in mutants missing the MexAB-OprM efflux pump or a component of the pump. Complementation studies showed that addition of the *mexAB-oprM* operon to deletion mutants restored the TTO and terpinen-4-ol susceptibility to that of the wild type, confirming the role of MexAB-OprM in tolerance to TTO and terpinen-4-ol. Interplay between the MexAB-OprM and MexCD-OprJ pumps may contribute toward tolerance of some components of TTO, including 1,8-cineole and α-terpineol. The possibility remains that other membrane pump systems not constitutively expressed, or not examined here, may contribute to the efflux of terpenes from *P. aeruginosa*. Nevertheless, this work further extends the already broad range of known substrates for MexAB-OprM to cyclic monoterpenes.

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REFERENCES

1. **Banes-Marshall, L., P. Cawley, and C. A. Phillips.** 2001. In vitro activity of *Melaleuca alternifolia* (tea tree) oil against bacterial and *Candida* spp. isolates from clinical specimens. Br. J. Biomed. Sci. **58:**139–145.
2. **Carson, C. F., K. A. Hammer, and T. V. Riley.** 2006. *Melaleuca alternifolia* (tea tree) oil: a review of antimicrobial and other medicinal properties. Clin. Microbiol. Rev. **19:**50–62.
3. **Carson, C. F., B. J. Mee, and T. V. Riley.** 2002. Mechanism of action of *Melaleuca alternifolia* (tea tree) oil on *Staphylococcus aureus* determined by time-kill, lysis, leakage, and salt tolerance assays and electron microscopy. Antimicrob. Agents Chemother. **46:**1914–1920.
4. **Choi, K. H., A. Kumar, and H. P. Schweizer.** 2005. A 10 min method for preparation of highly electrocompetent *Pseudomonas aeruginosa* cells: application for DNA fragment transfer between chromosomes and plasmid transformation. J. Microbiol. Methods **64:**391–397.
5. **Chuanchuen, R., K. Beinlich, T. T. Hoang, A. Becher, R. R. Karkhoff-Schweizer, and H. P. Schweizer.** 2001. Cross-resistance between triclosan and antibiotics in *Pseudomonas aeruginosa* is mediated by multidrug efflux pumps: exposure of a susceptible mutant strain to triclosan selects *nfxB* mutants overexpressing MexCD-OprJ. Antimicrob. Agents Chemother. **45:**428–432.
6. **Chuanchuen, R., R. R. Karkhoff-Schweizer, and H. P. Schweizer.** 2003. High-level triclosan resistance in *Pseudomonas aeruginosa* is solely a result of efflux. Am. J. Infect. Control **31:**124–127.
7. **CLSI.** 2006. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7–A7. CLSI, Wayne, PA.
8. **Coban, A. Y., B. Ekinci, and B. Durupinar.** 2004. A multidrug efflux pump inhibitor reduces fluoroquinolone resistance in *Pseudomonas aeruginosa* isolates. Chemotherapy **50:**22–26.
9. **Cox, S. D., and J. L. Markham.** 2007. Susceptibility and intrinsic tolerance of *Pseudomonas aeruginosa* to selected plant volatile compounds. J. Appl. Microbiol. **103:**930–936.
10. **De Kievit, T. R., M. D. Parkins, R. J. Gillis, R. Srikumar, H. Ceri, K. Poole, B. H. Iglewski, and D. G. Storey.** 2001. Multidrug efflux pumps: expression patterns and contribution to antibiotic resistance in *Pseudomonas aeruginosa* biofilms. Antimicrob. Agents Chemother. **45:**1761–1770.
11. **Driscoll, J. A., S. L. Brody, and M. H. Kollef.** 2007. The epidemiology, pathogenesis and treatment of *Pseudomonas aeruginosa* infections. Drugs **67:**351–368.
12. **Hancock, R. E.** 1998. Resistance mechanisms in *Pseudomonas aeruginosa* and other nonfermentative gram-negative bacteria. Clin. Infect. Dis. **27:**S93–S99.
13. **Hancock, R. E., and A. M. Carey.** 1979. Outer membrane of *Pseudomonas aeruginosa*: heat-2-mercaptoethanol-modifiable proteins. J. Bacteriol. **140:**902–910.
14. **Hirakata, Y., R. Srikumar, K. Poole, N. Gotoh, T. Suematsu, S. Kohno, S. Kamihira, R. E. Hancock, and D. P. Speert.** 2002. Multidrug efflux systems play an important role in the invasiveness of *Pseudomonas aeruginosa*. J. Exp. Med. **196:**109–118.
15. **International Organisation for Standardisation.** 2004. ISO 4730:2004, oil of *Melaleuca*, terpinen-4-ol type (tea tree oil). International Organisation for Standardisation, Geneva, Switzerland.
16. **Jiang, X., Z. Zhang, M. Li, D. Zhou, F. Ruan, and Y. Lu.** 2006. Detection of extended-spectrum β-lactamases in clinical isolates of *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **50:**2990–2995.
17. **Keen, N. T., S. Tamaki, D. Kobayashi, and D. Trollinger.** 1988. Improved broad-host-range plasmids for DNA cloning in gram-negative bacteria. Gene **70:**191–197.
18. **Lee, A., W. Mao, M. S. Warren, A. Mistry, K. Hoshino, R. Okumura, H. Ishida, and O. Lomovskaya.** 2000. Interplay between efflux pumps may provide either additive or multiplicative effects on drug resistance. J. Bacteriol. **182:**3142–3150.
19. **Li, X. Z., K. Poole, and H. Nikaido.** 2003. Contributions of MexAB-OprM and an EmrE homolog to intrinsic resistance of *Pseudomonas aeruginosa* to aminoglycosides and dyes. Antimicrob. Agents Chemother. **47:**27–33.
20. **Li, X. Z., L. Zhang, and K. Poole.** 1998. Role of the multidrug efflux systems of *Pseudomonas aeruginosa* in organic solvent tolerance. J. Bacteriol. **180:**2987–2991.
21. **Li, X. Z., L. Zhang, R. Srikumar, and K. Poole.** 1998. Beta-lactamase inhibitors are substrates for the multidrug efflux pumps of *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **42:**399–403.
22. **Longbottom, C. J., C. F. Carson, K. A. Hammer, B. J. Mee, and T. V. Riley.** 2004. Tolerance of *Pseudomonas aeruginosa* to *Melaleuca alternifolia* (tea tree) oil is associated with the outer membrane and energy-dependent cellular processes. J. Antimicrob. Chemother. **54:**386–392.
23. **Mann, C. M., S. D. Cox, and J. L. Markham.** 2000. The outer membrane of *Pseudomonas aeruginosa* NCTC 6749 contributes to its tolerance to the essential oil of *Melaleuca alternifolia* (tea tree oil). Lett. Appl. Microbiol. **30:**294–297.
24. **Masuda, N., E. Sakagawa, S. Ohya, N. Gotoh, H. Tsujimoto, and T. Nishino.** 2000. Contribution of the MexX-MexY-OprM efflux system to intrinsic resistance in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **44:**2242–2246.
25. **Messager, S., K. A. Hammer, C. F. Carson, and T. V. Riley.** 2005. Assessment of the antibacterial activity of tea tree oil using the European EN 1276 and EN 12054 standard suspension tests. J. Hosp. Infect. **59:**113–125.
26. **Okii, M., S. Iyobe, and S. Mitsuhashi.** 1983. Mapping of the gene specifying aminoglycoside 3′-phosphotransferase II on the *Pseudomonas aeruginosa* chromosome. J. Bacteriol. **155:**643–649.
27. **Papadopoulos, C. J., C. F. Carson, K. A. Hammer, and T. V. Riley.** 2006. Susceptibility of pseudomonads to *Melaleuca alternifolia* (tea tree) oil and components. J. Antimicrob. Chemother. **58:**449–451.
28. **Pearson, J. P., C. Van Delden, and B. H. Iglewski.** 1999. Active efflux and diffusion are involved in transport of *Pseudomonas aeruginosa* cell-to-cell signals. J. Bacteriol. **181:**1203–1210.
29. **Poole, K.** 2001. Multidrug efflux pumps and antimicrobial resistance in *Pseudomonas aeruginosa* and related organisms. J. Mol. Microbiol. Biotechnol. **3:**255–264.
30. **Schweizer, H. P.** 1998. Intrinsic resistance to inhibitors of fatty acid biosynthesis in *Pseudomonas aeruginosa* is due to efflux: application of a novel technique for generation of unmarked chromosomal mutations for the study of efflux systems. Antimicrob. Agents Chemother. **42:**394–398.
31. **Srikumar, R., T. Kon, N. Gotoh, and K. Poole.** 1998. Expression of *Pseudomonas aeruginosa* multidrug efflux pumps MexA-MexB-OprM and MexC-MexD-OprJ in a multidrug-sensitive *Escherichia coli* strain. Antimicrob. Agents Chemother. **42:**65–71.
32. **Srikumar, R., X. Z. Li, and K. Poole.** 1997. Inner membrane efflux components are responsible for beta-lactam specificity of multidrug efflux pumps in *Pseudomonas aeruginosa*. J. Bacteriol. **179:**7875–7881.